

**Amendments to the Specification.**

**Please add the priority information paragraph to the specification by inserting the following new paragraph before the first line of the specification:**

This is a continuation of application Serial No. 10/213,965 filed 7 August 2002, which is a continuation of application Serial No. 09/748,343 filed 22 December 2000, which is a continuation of application Serial No. 09/441,479 filed 17 November 1999 which is a divisional of application Serial No. 08/649,654 filed 13 May 1996 which is the §371 National Stage Entry of PCT/EP94/03801 filed 15 November 1994.

**Replace the paragraph beginning on page 4 line 15 with the following paragraph.**

Commercial MumpsVax virus was passaged on confluent monolayers of Vero cells grown in 25 cm<sup>2</sup> flasks with dMEM Biorich medium (50/50 v/v) with 0.5% foetal calf serum using about 3.0 log TCID<sub>50</sub> as inoculum. The infected cells were recovered after 7 days incubation at 34°C and the RNA extracted by the method of Ferré and Garduno (Nucleic Acids Research 1989, 17; 2141) into 100 mcl of water treated with diethylpyrocarbonate for 5 minutes at 100°C. 5 mcl of this extract was reverse transcribed by adding the following reagents: RNAsin 40 units (Boehringer Mannheim, Germany), 4mcl of 5X concentrated reverse transcriptase buffer (Bethesda Research Labs.), 2 mcl of a mixture of the four deoxynucleotide triphosphates at 10mM, 10 pmole of NH2 (SEQ ID NO:2) oligonucleotide primer, 1 mcl of Moloney murine leukemia virus (MMLV) reverse transcriptase (Bethesda Research Labs, 200 units per mcl) and water to a final volume of 20 mcl. Oligonucleotide NH2 has homology to the F gene of the Urabe strain of mumps virus. The mixture was incubated for 45 min at 37°C and then heated for 5 min at 95°C. The cDNA was then amplified by two successive rounds of PCR reaction using oligonucleotides NH8 (SEQ ID NO:3) and NH14 (SEQ ID NO:4) as primers and using 1 mcl of a thousand fold dilution of the first round reaction as starting material for the second round. Each PCR round consisted of 25 cycles of heating at 94°C for 1 min, 53°C for 1 min, 72°C for 1 min. The PCR product corresponding to the SH gene was sequenced in both directions after further PCR amplification in the presence of fluorodideoxynucleotide terminators and either NH8 or NH14 as primers and analysis of the products on an Applied Biosystems automatic (373A DNA sequencer) sequencer according to the suppliers protocol

and recommendations. Ambiguities were observed at a number of positions in the sequence and confirmed on both strands. The sequence obtained differed from that of Takeuchi et al (Virology 1991,181; 364-366) for Jeryl Lynn at 17 of 361 bases including 4 unassigned bases. The sequence further differed from part of that obtained by Afzal et al (J. Gen Virol. 1993, 74; 917-920) for their JL-5 isolate by 9 of 319 bases including 4 unassigned bases. Ambiguities were also observed when the same region was sequenced directly from 4.0 to 5.0 log TCID<sub>50</sub> MumpsVax virus recovered by ultracentrifugation, without prior passage on Vero cells, and after reverse transcription of viral RNA into cDNA with random primers followed by PCR amplification with oligonucleotides NH30bis (SEQ ID NO:9) and NH31bis (SEQ ID NO:10) as primers.

**Replace the paragraph beginning on page 5, line 12 with the following paragraph:**

MumpsVax virus was used to infect Vero cells and total RNA was prepared as described above. The RNA was reverse transcribed using random primers and PCR amplified using oligonucleotides NH22 (SEQ ID NO:5) and NH23 (SEQ ID NO:6) as primers. (NH22 contains a HindIII restriction site within the primer and NH23 contains a BamHI restriction site within the primer to facilitate cloning of the amplified DNA fragment). The amplified DNA was restricted with HindIII and BamHI endonucleases and cloned into the vector pUC9. Eleven clones containing an insert corresponding to the mumps SH gene region were recovered. All eleven had a sequence corresponding to that of Takeuchi et al (loc cit). In addition five clones had a DdeI restriction site, absent in the six other clones. No insert corresponding to the JL-5 sequence was recovered. This result and the sequencing ambiguities suggested the the JL-2 variant virus defined by Afzal et al forms a substantial or easily detectable proportion of MumpsVax virus.

**Replace the paragraph beginning on page 7 line 16 with the following paragraph:**

To attempt to determine more directly the proportion of the JL-5 and JL-2 type variants in MumpsVax and derivative cultures a plaque hybridization method was used. MumpsVax virus and the passaged virus of lot MJ05 were used to infect Vero cell monolayers and obtain plaques which were then

lifted onto nylon membranes and the nucleic acids fixed as described above. The filters were prehybridized for 3 hours at 65°C in 200 ml of the following solution: 5X SSC (SSC is 0.15M sodium chloride 0.01M sodium citrate pH 7.2), (10X concentrated Denhardt's solution (10X concentrated Denhardt's solution is : 0.2% w/v Ficoll 400, 0.2% bovine serum albumin, 0.2% polyvinyl chloride), 0.1% (w/v) sodium dodecyl sulphate, Salmon sperm DNA 50 mcg per ml. The filters were then hybridized with gentle agitation for 2.5 hours at 65°C in 50 ml of a solution with the same composition as above and preheated to 65°C and with the addition of the radioactive probe and cold competitor probe solution. The oligonucleotides used as variant specific probes were BC252 (SEQ ID NO:12) which hybridizes with JL-5 variants and BC253 (SEQ ID NO:13) which hybridizes with JL-2 variants. The oligonucleotides were labelled with gamma <sup>32</sup>P-ATP by kination in a solution of the following composition: 100ng of the oligonucleotide to be labelled, 3 mcl of 10X concentrated kinase buffer (10x concentrated kinase buffer contains: 0.5M tris -HCl pH 7.6, 0.1M MgCl<sub>2</sub>, 50mM dithiothreitol. 1mM spermidine and 1mM EDTA pH 8.0), 3 mcl of <sup>32</sup>P- ATP (Amersham International, 3000 Ci/nmole, 10mCi/mcl) and 2 mcl of T4 polynucleotide kinase (Boehringer Mannheim,) made up to 30mcl with sterile water. This mixture was incubated for 30 minutes at 37°C and the reaction stopped by heating for 5 minutes at 95°C. Cold competitor oligonucleotide was then added at a (w/w) ratio of 100 to 1, that is 10 mcg of cold competitor oligonucleotide was added for every 100 ng of labelled probe, before adding the mixture to the hybridization solution. After hybridization the filters were washed once for 30 minutes at 65°C in 100 ml of a solution with the same composition as the hybridization solution and then washed at 65°C in two changes of 100 ml of a solution of the following composition: SSC 5X, 0.1% sodium dodecyl sulphate. The filters were then dried and exposed to X-ray film with an intensifying screen. When Mumps Vax was examined by this technique a large excess of plaques hybridized with oligonucleotide BC252 specific for the JL-5 variant compared to the hybridization found with BC253. When lot MJ05 was examined, although there were approximately equal numbers of plaques hybridizing with both probes, relatively more plaques hybridized with BC253 than with BC252.

**Replace the paragraph beginning on page 9 line 14 with the following paragraph:**

One virus isolate, originating from well 9H2A of the micro titre plate and further identified as SBB strain JL-1 was taken through two further passages on CEF cells. After the last passage (4 passages from the original Mumps Vax material), the virus was used to infect Vero cells and to obtain plaques. These were lifted onto nylon membranes and tested by hybridization with oligonucleotides BC252 and BC253 which had been labelled with <sup>32</sup>P by kination. Over 2000 plaques were tested with the JL-2 specific probe BC253 and none was found to react with this. A lesser number of plaques was tested with oligonucleotide BC252 and all gave a positive reaction. Sequencing was performed directly on the virus pool of the JL-1 strain recovered at the fourth passage on CEF cells by centrifugation and ethanol precipitation of the virus followed by reverse transcription using random primers as described above. The cDNA was amplified by PCR reaction using oligonucleotides NH14 and BC265 (SEQ ID NO:11) as primers and with the following heating programme: 1 minute at 94°C, 1 minute at 60°C, 1 minute at 72°C, for 30 cycles. The resulting DNA fragment was purified on a MagicPrep column (Promega Biotech) according to the suppliers' instructions and sequenced on an Applied Biosystems 373A automatic sequencer according to the manufacturers' instructions and using oligonucleotides NH14, BC 265, NH 30bis and NH31bis as primers. The sequence shown in Figure 1 was obtained. This sequence surprisingly differs from that obtained by Afzal et al for their JL-5 isolate at six positions in the intergenic region between the SH and HN coding regions as shown in Figure 2. The JL-1 isolate therefore represents a further variant virus present in the MumpsVax preparation.

**An Abstract on a separate sheet is attached as required under 37 CFR 1.72(b). Please insert the attached abstract, following the claims.**